networks through a set of biochemical reactions is generally thought to predict an average kinetic behavior. Stochastic kinetic methods, accounting for random fluctuations, become essential to characterize intrinsic heterogeneity due to low copy numbers and noisy environments present in these systems. While in certain regulatory constructs, it has been shown that a fully discrete stochastic treatment of fluctuations through the Chemical Master Equation yields multi-stability when deterministic treatment predicts monostability; deterministic or continuous approaches continues to be the most widespread method for modeling the behavior of cell populations. We explore stochastic variability in cellular dynamics through a varied treatment of intrinsic noise, the randomness arising either at the level of a single reaction, single molecule or as a drift in concentration of the involved species. Our study involves modeling of a genetic toggle switch which is a set of two mutually repressing genes, acting as a cellular ''memory device'' during cell differentiation process, deciding the final cell fate. We simulate the complex switching dynamics between the multi-attractor states in these systems through direct sampling using continuous stochastic approaches Langevin dynamics and Fokker Plank equation as well as discrete stochastic methods Gillespie's Stochastic Simulation Algorithm and numerical solution of the Chemical Master Equation. The stochastic trajectories obtained through numerical simulations not only unveil the complex dynamics leading to multi-stability, they also help explore rigorously the variations, if any, in the dynamics of transitions. Our results can further be used to compare the statistics of switching events and hence to benchmark the various computational methods available to model stochasticity in such systems.

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Optimizing Protein Expression Levels as a Function of Network Topology Minimizes Nonfunctional Complex Formation

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Human cells contain ~20,000 genes encoding upwards to 100,000 protein types. 5-40% of cell volume is occupied by macromolecules, posing challenges for cell proteins to locate functional partners and avoid nonspecific interactions. Overexpressed proteins may saturate functional partners, leaving leftovers for nonspecific binding instead. Eukaryotic cells have evolved various methods to help proteins function reliably, including compartmentalization, allostery, and structural properties of binding sites. In addition to these, cells may optimize protein expression levels as a function of network topology to avoid nonspecific binding.

To study the effects of relative protein abundance on nonspecific complex formation, we first simulated five simple network motifs under varying protein concentrations using the Gillespie algorithm. While the motifs formed roughly the same proportion of nonspecific interactions under optimal conditions, they varied in sensitivity to initial concentrations (ICs), with the hub being the most sensitive and triangle being the least. We then simulated 500 large networks of 90-200 nodes with varying topological properties under equal, random, and optimized ICs. Binding affinities for all specific and nonspecific interactions were determined using a coarse-grained protein sequence model. The proportion of protein in nonspecific complexes was recorded as a function of degree distribution, network density, average binding strength, local topology, and ICs. It was found that optimizing the local topology via introducing more hubs and less chains and flags; similar to real networks; decreased the number of nonspecific complexes under optimal ICs, but also increased sensitivity to ICs. Degree distribution, surprisingly, had little influence once local topology was optimized. We conclude that there is evolutionary pressure to both favor certain network motifs and to balance protein abundance to avoid misinteractions.

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Designing Stem-Cell Based Anti-HIV Therapies using Molecular-Detailed Multiscale Models

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Although HAART ensures normal lives for HIV-infected individuals, it does not lead to a functional cure. In 2008, the ''Berlin patient'', a HIV-infected cancer patient, underwent a bone marrow transplant from a CCR5-/- donor. Since then, he has shown no signs of active HIV-1 replication in the absence of HAART. However, finding such a matched donor for each HIV patient is challenging; instead, inserting anti-HIV genes into patients' or matched donors' stem cells before transplantation could provide HIV-resistance to the progeny target cells and lead to a functional cure.

We developed a molecular-detailed, mechanistic model of HIV infection to design stem-cell therapies using endogenous anti-HIV genes and to identify logistics of successful treatment. The model simulates the dynamics of HIV infection to track key restriction factors and their interactions with HIV-encoded proteins, the virus, and multiple immune cell types: CD4+ T-cells, macrophages, latently-infected T-cells, and CTLs. Using viral load and T-cell count data from HIV-infected individuals, we explored interpatient variability of stem-cell therapies by creating a virtual population of HIV patients. Our model predicted that APOBEC3G overexpression at medium or high transduction efficiency could effectively stop HIV replication and increase the T-cell count to normal levels. SAMHD1 overexpression in macrophages even at high transduction efficiency is not by itself a potent inhibitor of HIV replication; it stabilizes the viral load and slightly improves the T-cell count. However, the model predicted APOBEC3G-SAMHD1 synergy: faster viral decay and return to normal T-cell counts at lower overexpression levels. Surprisingly, HIV-triggered pro-apoptosis gene circuits combined with APOBEC3G overexpression were predicted to negatively impact HIV replication blockade if expressed at low transduction efficiency. This is because some infected T-cells die at a faster rate, weakening the effect of immune response in killing the remaining infected cells.

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DNA Fluorescence Parameters and Methylation Levels of Gut Commensal Escherichia coli from Crohn's Disease Patients

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Previously it was shown by us the high concentrations of E. coli in feces of Crohn's disease (CD) patients. The differences in growth of gut commensal E. coli both in aerobic and anaerobic conditions, was also demonstrated [1, 2]. Taking into account the preliminary results on DNA methylation levels and the possible impact of DNA structure on DNA fluorescence parameters, the goal of current investigations was to study the DNA fluorescence parameters of commensal E. coli isolates from the gut microbiota of CD patients in association with the DNA methylation levels.

10 CD patients and 10 healthy persons were involved in this study, and at least, 5 randomly selected gut E. coli isolates from each healthy and diseased person were investigated.

Comparative analysis has shown that DNA methylation levels in patients' E. coli were significantly higher than in E. coli of healthy individuals, and the levels correlated with the duration and stage of the disease. The differences in fluorescence parameters of DNA from CD patients' gut E. coli isolates were also revealed by us compared with the control samples of healthy people. Chronic inflammation induces a cascade of pro-inflammatory and anti-inflammatory molecules. The balance between these two groups of regulators controls cell death and repair of tissue damage. In recent years it has become apparent that gut commensals produce molecules which can counteract pro-inflammatory and anti-inflammatory pathways leading to activation or repression of immunity genes.

The above mentioned data suggests that epigenetic gene control mechanisms can be involved in bacterial induced or supported inflammation during CD.

References:

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Isolation, Fragmentation and the Detection of Listeria DNA from Ground Beef

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Listeria is a gram-positive, rod shaped food borne bacterial pathogen with a mortality of 20-30% of those who get infected. People with a compromised immune system and pregnant women are more likely to suffer effects of Listeriosis.[1] Listeria bacterium can grow under extreme conditions such as low pH and high temperatures. Places that are most likely to transmit the bacterium are food processing plants during any food processing step. Once Listeria is contracted in the human body, it can cross in to the blood, through the blood brain barrier, and into the cerebral spinal fluid. Current detection such as PCR methods are slow and costly and the extraction methods to see if Listeria is present is blood work, spinal tap, or a biopsy of the placenta.

In this paper we show that Listeria DNA can be efficiently and rapidly extracted from ground bovine meat and lysed. Our microwave based lysing approach[2] has particular advantages in that it can fragment the Listeria genome to smaller